September 20, 1976

Dr. Paul Berg
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Dear Paul:

I am sending separately the following materials you requested:

SV40: dl-1001, 1002, 1009
Cells: Balb/c 3T3

dl-1001 is a stable mutant, but 1002 and 1009 have repeatedly given other variants when cloned and propagated. dl-1002 DNA can be readily purified by gel electrophoresis; dl-1009 DNA, with more difficulty, and we have therefore not done anything with it. All of these were cloned with tsB helper. To make stocks we apply 0.2 ml of a 1:10 dilution of original stock (made from a plaque suspension) to the edge of a 10-cm cell monolayer, let virus absorb at 37° for 60', and incubate at 40° until lysis.

Balb/c 3T3 cells came from George Todaro in December, 1974, as his A31 subclone 7, the most contact-inhibited clone he had. Still one gets spontaneous transformants when these cells are passed for any length of time. I suggest you freeze down an early passage and of course check what you grow for spontaneous morphologic transformation. We noticed that SV40 transformants of Balb/c 3T3 are much denser than spontaneous ones and all colonies of this type we tested (about two dozen) had SV40 T antigen. I have also used secondary CHL cells (Chinese hamster lung cells) obtained from Bob Martin and Janice Chou. SV40 transformants were easy to score, and the cells could be transformed by SV40 DNA. We have not tried rat cells.

The enclosed table gives results of transformation of CHL and Balb/c 3T3 cells by SV40 DNA with Graham and van der Eb's procedure. We have not had a great deal of experience with the DNA transformations, and specifically we have not varied conditions (for example, plating smaller numbers of infected cells) to see if we could get a more linear dose response.

In regard to dl-1003, missing Hin-E, Ching-Juh Lai found that it does not complement tsB, C or D mutants (see his 1974 Cold Spring Harbor paper and enclosed preprint), from which we inferred that Hin-E may have

a transcriptional, processing, or translational signal for expression of the B/C gene. Ching-Juh is now following this up with George Khoury along the lines you outlined in your letter.

As far as our plans for early deletion mitants go, Stu Adler now has a fairly complete set, and he plans to test them for biological properties, including early mRNA and T antigen protein. I think at the present stage our labs can't avoid overlaps like this, since the mutants are mostly new, but I too hope we can exchange information and materials to cut down undesirable duplication. I expect we'll be branching off in different directions before very long.

Thanks very much for the invitation to visit Stanford. I would like to come, but in the coming months I'll be away from the lab a fair amount and am reluctant to increase the time away. Please give me a rain check.

Let me know if there is a problem with cells or virus when they arrive. I look forward to seeing you in Miami.

Sincerely,

Daniel Nathans

DN:as
Enc.

SV40 DNA transformation

µg DNA I	Transformed colonies CH2	Balb 3T3
0	0, 0	0, 0, 0, 0
0.1	5, 3	0, 0
0.2	14, 7	8, 12
0.5	17, 15	10, 5
1.0	24, 16, 20, 25	9, 10
2.0	50, —	15, 21, 20, 13

~10^5 cells in 2 cm wells, just confluent: CH2 grown in MEM + 10% FBS; Balb 3T3 grown in MEM + 20% FBS.
Add DNA suspension in 0.1 ml volume — ρ removal of medium
Incubate at room temperature for 20'
Do not remove DNA suspension
To CH2 add 1 ml MEM + 5% FBS
To 3T3 " " " " 10% FBS
Incubate overnight at 37° in CO_2 incubator (~ 15 hrs)

Next morning remove medium and transfer cells to a 10 cm dish (from each 2 cm well), using MEM + 5% FBS in all cases.

Score piled up colonies after 3 wks.

DNA solution, made immediately before use exactly as in Graham & van der Eb, Virol. 52, 456-467, 1973.